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Article Addendum

New insights into the therapeutic inhibition of voltage-gated sodium channels

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Antiarrhythmics, anticonvulsants and local anesthetics inhibit voltage-gated sodium channels and reduce membrane excitability in neurons and muscle, making them useful in the management of cardiac arrhythmias, epilepsy and pain. These compounds, which are often termed singly in the literature as 'local anesthetics', have at least two inhibitory states: a resting inhibition that develops with intermittent stimulation and a higher affinity inhibition that arises upon repeated depolarization and likely involves the inactivated state of the channel. Although elucidating their mechanism of inhibition has been an active area of research for decades, many questions remain unanswered. Do these two inhibitory states share a common, but guarded or modulated receptor? Or do they represent different protonated states of the drugs, many of which have pK_a's close to physiological pH, thereby yielding a significant population of both charged and uncharged compound inside cells. Some mechanistic clues can be found by mutating conserved phenylalanine and tyrosine residues of the 'local anesthetic receptor' in the channel's inner vestibule. Mutations of these aromatic residues universally disrupt the mechanism of drug inhibition in numerous channel isoforms. For instance, non aromatic substitutions of Phe1579 (Na_v1.4 numbering) in the pore lining S6 segment of domain four (DIVS6) can abolish inactivated state inhibition.^{1,2} The strict conservation of Phe1579 and other DIVS6 aromatic residues in all nine sodium channel isoforms led us to further dissect the role of this and other aromatic residues on local anesthetic inhibition. We recently employed subtly modified

phenylalanine derivatives to better understand the role of these aromatics in the binding of local anesthetics and found a significant electrostatic interaction at one site, Phe1579, contributes to channel inhibition.³ What follows is a self guided tour of our motivation and experimental findings.

Aromatic Side Chains Can Make Cation- π Interactions with Cationic Ligands

We set out to test the possibility that a cation- π interaction between cationic local anesthetics and the negative electrostatic potential on the face of the aromatic rings of phenylalanine or tyrosine contributes to the inhibitory mechanism. These residues are especially suited to bind organic cations because the energy of dehydrating the aromatic and the blocker are well matched,⁴ thus allowing the blocker to get close to the face of the aromatic. Evidence for a cation- π interaction can also be insightful structurally as these interactions occur only when the cation is oriented toward the face, not the edge, of the aromatic ring.⁵ This prerequisite differs from the electrostatic interactions between the monopole charges of the side chains of glutamate or aspartate and lysine or arginine. In the latter case sterics plays less of a role. We have shown previously that cation- π interactions support the binding of cationic ligands to their aromatic lined receptors in GABA, acetylcholine and 5HT channels,⁶⁻⁹ and the extracellular block of voltage-gated sodium channels by tetrodotoxin (TTX) and Ca²⁺ and potassium channels by tetraethylammonium (TEA).¹⁰⁻¹² In these examples, serial fluorination of the aromatic side chain is used to empirically determine the energetic contribution of a cation- π interaction to binding. With this method, one, two or three fluorines are appended to the aromatic ring and serve to reduce the negative electrostatic potential on the face of the side chain in a step wise and predictable fashion while leaving the other chemical attributes of the side chain unaffected, including hydrophobicity.^{5,13}

A Single Cation- π Interaction Underlies Use-Dependence

Using the in vivo nonsense suppression method,¹⁴ we incorporated serially fluorinated phenylalanine residues at three aromatic sites in the Na_v1.4 skeletal muscle sodium channel. Mutation of the sites chosen, Phe1574, Phe1579 and Tyr1586 was shown previously to affect channel inhibition by local anesthetics.^{2,15,16} The fluoro

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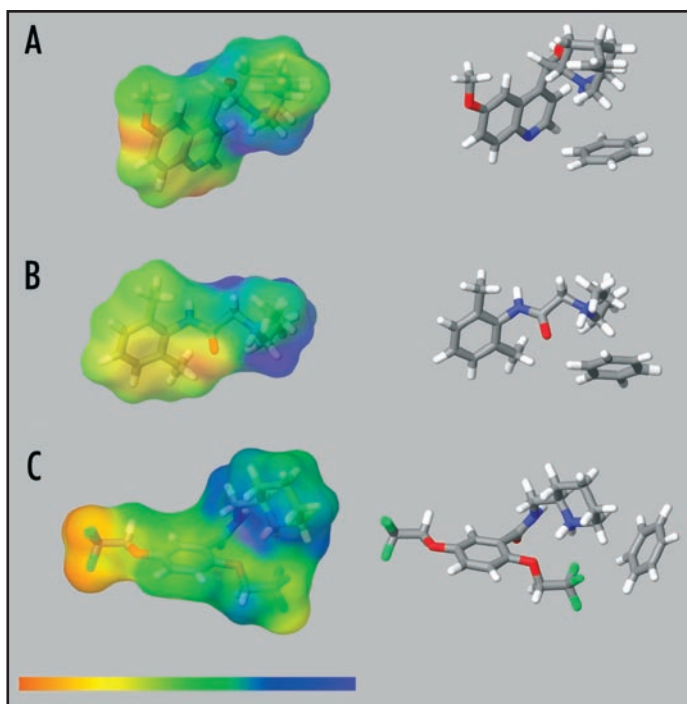


Figure 1. Diversity of electrostatic potential distribution and coordination chemistry with benzene amongst Class 1 anti-arrhythmic compounds. Analysis of quinidine, lidocaine and flecainide are shown in panels (A–C), respectively. Left hand panels show the electrostatic potential calculated at the surface generated by a 2 Å spherical probe (gas phase, B3LYP/6-31+G**). Right hand panels show the optimized binding interactions with benzene (B3LYP/6-31G*). Quantum mechanical calculations used Jaguar (www.Schrodinger.com). Scale bar shows the color coded range of potentials from 0 (red) to +140 kcal/mol (blue). In each case, benzene interacts preferably with the more positively charged regions of the drug.

Phe derivatives at these sites were well-tolerated and produced channels with robust expression and normal voltage dependence of gating, substantially simplifying the interpretation of their effects on drug binding. Tonic block of channels by the local anesthetic and Class 1B antiarrhythmic compound lidocaine at 200 μ M was assessed with 15 ms depolarizing pulses delivered at 20 s (0.05 Hz) intervals. Under these ‘resting’ conditions, fluorination at these sites had no effect on block indicating no electrostatic interaction between the aromatic ring and the drug. At higher frequencies, up to 20 Hz, fluorination disrupted use dependent inhibition at only one site, Phe1579, indicating that a significant cation- π interaction between the drug and this aromatic underlies use dependent inhibition. The identical manipulation at the two other sites, Phe1574 or Phe1586, had no effect.

The Role of Charge

Might the lack of an electrostatic interaction in the resting state between the lidocaine and the aromatics be due, in part, to the protonation state of the drug? For instance, lidocaine could be in close proximity to the face of the tested aromatic, but in a neutral form during resting inhibition. Indeed, inhibition by the neutral local anesthetic benzocaine was similarly unaffected by fluorination, suggesting not only that these mutations primarily affect the electrostatic properties of the aromatic side chain, but also that neutral blockers are oblivious to these manipulations. To answer the above

question directly, we investigated block with QX 314, a drug with a similar structure as lidocaine except that it carries a permanent cationic charge on its quaternary ammonium moiety. For these experiments, we chose to tri fluorinate a single site, Phe1579, both because of its central location in the local anesthetic receptor and because this manipulation slightly inverts the quadrupole moment of the aromatic ring.¹² Surprisingly, even ablating the aromatic negative potential at Phe1579 by tri-fluorination had no effect on the resting state blockade of QX 314, demonstrating that the blocker, while fully charged, does not have electrostatic access to Phe1579 in the tonic state. Why then does Tyr1586, which lies roughly on the same face of the S6 helix as Phe1579, lack a measurable cation- π interaction in any state? One possibility is that the blocker might bind deep within the inner vestibule, perhaps plastered up against the selectivity filter, thus putting it out of reach from this distal S6 residue.¹

Putting It All Together

What new mechanistic insights can be drawn from this work? Previous experiments have suggested that the voltage dependence of the resting and stimulated states is similar, implying that the location of the charged compound in relation to the membrane electric field does not change when channels are stimulated.¹⁷ In terms of resting block, might the blocker be in the inner vestibule but in a position where it would not interact with Phe1579? We believe that this possibility is unlikely. Although the positive charge of the local anesthetic is concentrated near the ammonium group of the compound, the entire molecule carries a positive electrostatic charge (Fig. 1). Thus, in the tight quarters of the inner vestibule any manipulation of the electrostatic potential on the face of the aromatic ring, which reaches ~ 600 mV at a distance of 2 Å from the center of the aromatic ring,¹² should produce an effect if the aromatic is facing the inner vestibule. Our results therefore support the notion that the local anesthetic resides in the inner vestibule of the channel during resting or tonic blockade. Apparently at this stage the charged form of the drug is not a necessary requisite for use dependent inhibition, primarily because Phe1579 is not facing the permeation pathway of the channel. Upon repeated stimulation, Phe1579 presents its aromatic side chain towards the charged blocker (perhaps by rotation)¹⁸ with a movement we hypothesize to be concomitant with channel inactivation. We readily acknowledge that reorientation of Phe1579 is only one of a number of protein rearrangements which likely underlie channel inactivation, a process that also involves the cytosolic III-IV linker and residues in other domains. Once bound, in part utilizing a cation- π interaction, the drug serves to hold Phe1579 in the inner vestibule, stabilizing both the inactivated state of the channel and possibly, the activated voltage sensors in nearby domains.¹⁹

Future Directions

Diversity of charge distribution on Class 1 anti-arrhythmic drugs. Given the importance that cationic charge on the drug plays in the mechanism of use dependent inhibition, we investigated how it is distributed on three Class 1 drugs. For this analysis we chose quinidine, lidocaine and flecainide, each a representative of a Class 1 subcategory, A, B or C, respectively, and generated electrostatic potential distributions using ab initio quantum mechanical calculations. The results of this analysis are seen in figure one (left-hand panels) and demonstrate that significant diversity of

charge distribution exists among these sodium channel inhibitors, leading to divergent interaction stereochemistry with benzene (right-hand panels). In fact, although the entire surface of these compounds present a delocalized positive electrostatic potential, the more positively charged regions (blue) attract benzene more strongly. Future experiments will determine if these differences will translate into mechanistic heterogeneity that could be exploited in the design and discovery of novel inhibitors.

The Achilles heel of local anesthetics as clinical tools in the management of membrane excitability is their lack of isoform specificity, owing mostly to the conserved nature of their receptor in the channel's inner vestibule. Thus, the more we can learn about the details of the state dependent interactions between the drug and the channel, the closer we will be to designing drugs that can exploit the subtle differences in blocking mechanisms between isoforms. The experiments described here put us one step closer to that distant goal.

References

- McNulty MM, Edgerton GB, Shah RD, Hanck DA, Fozzard HA, Lipkind GM. Charge at the lidocaine binding site residue Phe 1759 affects permeation in human cardiac voltage-gated sodium channels. *J Physiol* 2007; 581:741-55.
- Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Molecular determinants of state dependent block of Na⁺ channels by local anesthetics. *Science* 1994; 265:1724-8.
- Ahern CA, Eastwood AL, Dougherty DA, Horn R. Electrostatic contributions of aromatic residues in the local anesthetic receptor of voltage-gated sodium channels. *Circ Res* 2008; 102:86-94.
- Gallivan JP, Dougherty DA. A computational study of cation- π interactions vs salt bridges in aqueous media: Implications for protein engineering. *J Am Chem Soc* 2000; 122:870-4.
- Ma JC, Dougherty DA. The cation- π interaction. *Chem Rev* 1997; 97:1303-24.
- Zhong WG, Gallivan JP, Zhang YN, Li LT, Lester HA, Dougherty DA. From *ab initio* quantum mechanics to molecular neurobiology: A cation- π binding site in the nicotinic receptor. *Proc Natl Acad Sci* 1998; 95:12088-93.
- Beene DL, Brandt GS, Zhong W, Zacharias NM, Lester HA, Dougherty DA. Cation π interactions in ligand recognition by serotonergic (5 HT_{3A}) and nicotinic acetylcholine receptors: The anomalous binding properties of nicotine. *Biochem* 2002; 41:10262-9.
- Lummis SC, Beene DL, Harrison NL, Lester HA, Dougherty DA. A cation- π binding interaction with a tyrosine in the binding site of the GABA_A receptor. *Chemistry and Biology* 2005; 12:993-7.
- Padgett CL, Hanck AP, Lester HA, Dougherty DA, Lummis SC. Unnatural amino acid mutagenesis of the GABA(A) receptor binding site residues reveals a novel cation π interaction between GABA and β 2Tyr97. *J Neurosci* 2007; 27:886-92.
- Ahern CA, Eastwood AL, Lester HA, Dougherty DA, Horn R. A cation- π interaction between extracellular TEA and an aromatic residue in potassium channels. *J Gen Physiol* 2006; 128:649-57.
- Santarelli VP, Eastwood AL, Dougherty DA, Horn R, Ahern CA. A cation- π interaction discriminates among sodium channels that are either sensitive or resistant to tetrodotoxin block. *J Biol Chem* 2007; 282:8044-51.
- Santarelli VP, Eastwood AL, Dougherty DA, Ahern CA, Horn R. Calcium block of single sodium channels. Role of a pore lining aromatic residue. *Biophys J* 2007; 93:2341-9.
- Flocco MM, Mowbray SL. Planar stacking interactions of arginine and aromatic side chains in proteins. *J Mol Biol* 1994; 235:709-17.
- Nowak MW, Kearney PC, Sampson JR, Saks ME, Labarca CG, Silverman SK, Zhong W, Thorson J, Abelson JN, Davidson N, Schultz PG, Dougherty DA, Lester HA. Nicotinic receptor binding site probed with unnatural amino acid incorporation in intact cells. *Science* 1995; 268:439-42.
- Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Common molecular determinants of local anesthetic, antiarrhythmic, and anticonvulsant block of voltage-gated Na⁺ channels. *Proc Natl Acad Sci* 1996; 93:9270-5.
- Liu G, Yarov-Yarovoy V, Nobbs M, Clare JJ, Scheuer T, Catterall WA. Differential interactions of lamotrigine and related drugs with transmembrane segment IVS6 of voltage-gated sodium channels. *Neuropharmacology* 2003; 44:413-22.
- Gingrich KJ, Beardsley D, Yue DT. Ultra deep blockade of Na⁺ channels by a quaternary ammonium ion: Catalysis by a transition intermediate state? *J Physiol* 1993; 471:319-41.
- Sunami A, Tracey A, Glaaser IW, Lipkind GM, Hanck DA, Fozzard HA. Accessibility of mid segment domain IVS6 residues of the voltage-gated Na⁺ channel to methanethiosulfonate reagents. *J Physiol* 2004; 561:403-13.
- Hanck DA, Makielski JC, Sheets MF. Lidocaine alters activation gating of cardiac Na channels. *Pflugers Archiv Eur J Physiol* 2000; 439:814-21.